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Protective role of 2',6'- dihydroxyacetophenone in mitigating diabetes- driven hepatic injury in rats

Papel protetor da 2',6'-di-hidroxiacetofenona na mitigação da lesão hepática induzida pelo diabetes em ratos

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ABSTRACT

Background: Naturally derived compounds with antioxidant and hepatoprotective potential are being actively explored. 2′,6′-Dihydroxyacetophenone (DHDA), a phenolic compound, has shown antioxidant properties, but its role in diabetic liver injury remains inadequately investigated. **Aim and objectives:** This study aimed to assess the protective effects of DHDA on diabetes-induced liver injury in a rat model. **Materials and methods: Acute toxicity was conducted by using mice.** Diabetes was induced in Wistar rats using streptozotocin (STZ) following nicotinamide(NA) pretreatment. Animals were divided into four groups: normal control, diabetic control, DHDA at dose of 30 mg/kg, and DHDA at dose 60 mg/kg. DHDA was administered orally for three weeks. Fasting blood glucose (FBG), body weight, food intake and, water intake were evaluated. In addition, the biochemical parameters, *in-Vitro* antioxidant and the histopathology of liver were analyzed. **Result: 300 mg/kg is the safe dose of DHDA.** DHDA significantly reduced FBG and glycated haemoglobin (HbA1c) levels in diabetic rats. Elevated liver enzymes and oxidative stress markers were markedly attenuated, while endogenous antioxidant defences were restored, particularly at dose of 60 mg/kg. *In vitro* assays confirmed dose-dependent free radical scavenging activity. Histopathological examination showed notable improvement in hepatic architecture. **Conclusion:** DHDA exhibits significant antihyperglycaemic, antioxidant, and hepatoprotective effects in diabetic rats, highlighting its potential as a therapeutic candidate for diabetes-associated liver injury.

Keywords: diabetes mellitus; hepatic injury; oxidative stress; 2',6'-dihydroxyacetophenone; antioxidant therapy.

RESUMO

Contexto: Compostos de origem natural com potencial antioxidante e hepatoprotetor estão sendo ativamente explorados. A 2′,6′-diidroxiacetofenona (DHDA), um composto fenólico, demonstrou propriedades antioxidantes, mas seu papel na lesão hepática diabética permanece insuficientemente investigado. **Objetivo:** Este estudo teve como objetivo avaliar os efeitos protetores da DHDA na lesão hepática induzida por diabetes em um modelo de rato. **Materiais e métodos:** O estudo de toxicidade aguda foi realizado em camundongos.O diabetes foi induzido em ratos Wistar utilizando estreptozotocina (STZ) após pré-tratamento com nicotinamida (NA). Os animais foram divididos em quatro grupos: controle normal, controle diabético, DHDA na dose de 30 mg/kg e DHDA na dose de 60 mg/kg. A DHDA foi administrada por via oral durante três semanas. Foram avaliados a glicemia de jejum

(GJ), o peso corporal, a ingestão de alimentos e a ingestão de água. Além disso, foram analisados os parâmetros bioquímicos, a atividade antioxidante in vitro e a histopatologia do fígado. **Resultado:** A dose de 300 mg/kg foi considerada segura para o DHDA.O DHDA reduziu significativamente os níveis de glicemia de jejum (GJ) e hemoglobina glicada (HbA1c) em ratos diabéticos. As enzimas hepáticas elevadas e os marcadores de estresse oxidativo foram acentuadamente atenuados, enquanto as defesas antioxidantes endógenas foram restauradas, particularmente na dose de 60 mg/kg. Ensaios in vitro confirmaram a atividade de eliminação de radicais livres dependente da dose. O exame histopatológico mostrou melhora notável na arquitetura hepática. **Conclusão:** O DHDA exibe efeitos anti-hiperglicêmicos, antioxidantes e hepatoprotetores significativos em ratos diabéticos, destacando seu potencial como candidato terapêutico para lesão hepática associada ao diabetes.

Palavras-chave: diabetes mellitus; lesão hepática; estresse oxidativo; 2',6'-diidroxiacetofenona; terapia antioxidante

INTRODUCTION

Diabetes Mellitus remains one of the most pressing global health challenges, with its prevalence rising steadily across developing and developed nations. Over the past three decades, the number of adults with diabetes has almost doubled due to changes in lifestyle, urbanization, and longer life expectancies. (1) The great majority of cases are type 2 diabetes, which is characterized by persistent hyperglycemia brought on by either insulin resistance, decreased insulin production, or a combination of the two. Diabetes‘s metabolic impact goes well beyond glucose dysregulation and significantly impacts essential organs, including the liver, even though its clinical consequences mostly focus on vascular problems. Because the liver is essential to both lipid metabolism and glucose homeostasis, it is especially susceptible to long-term metabolic problems linked to diabetes. Hepatic tissues undergo biochemical and histological changes as a result of persistent hyperglycemia‘s promotion of oxidative stress, dyslipidemia, and inflammatory reactions (2). As a result, diabetic hepatopathy which includes cirrhosis, fibrosis, steatohepatitis, and non-alcoholic fatty liver disease (NAFLD) is becoming more well acknowledged as a prevalent comorbidity among diabetes patients (3). Hepatic problems emerge early in the course of diabetes and may grow silently before overt clinical signs appear, according to emerging clinical and experimental data. Despite these results, the majority of current treatment plans concentrate on glycaemic management and provide little defense against liver damage caused by diabetes.(3)

Many people believe that oxidative stress plays a key role in the pathophysiology of diabetic liver damage. Inadequate antioxidant defence combined with excessive production of reactive oxygen species (ROS) causes lipid peroxidation, DNA damage, protein denaturation, and mitochondrial dysfunction, all of which compromise cellular integrity. This redox imbalance contributes to liver damage and fibrosis by triggering fibrogenic pathways and pro-inflammatory cytokines [4-6]. Hepatic structural and functional degradation is also accelerated by persistent low-grade inflammation and altered intracellular signaling [7,8]. As a result, methods that address inflammation and oxidative stress may present viable treatment options to lessen hepatic damage brought on by diabetes. Because of their hepatoprotective, anti-inflammatory, and antioxidant qualities, natural products and bioactive chemicals produced from plants have drawn more and more interest from researchers as possible treatment options [6]. Particularly well-known for their potent capacity to scavenge free radicals and alter cellular signaling pathways linked to oxidative stress are phenolic chemicals. Additionally, they show promise for enhancing mitochondrial function, lipid metabolism, and insulin sensitivity. DHDA, a phenolic derivative that has been isolated, commonly found in Rutaceae like *E.merrillii [7]* , and is one of these substances that has lately come to light as a potential bioactive molecule with pharmacological significance.

According to preliminary biochemical and molecular research, DHDA modulates both enzymatic and non-enzymatic defense mechanisms to demonstrate strong antioxidant activity. Its structural characteristics allow it to effectively neutralize free

radicals and donate hydrogen atoms [8]. Furthermore, DHDA present an antitumor activity against mTOR mediated colorectal cancer [9]. Comprehensive *in vivo* evaluations of DHDA in metabolic illness models, particularly those associated with diabetic liver injury, are still few, despite encouraging in *vitro* data.

Diabetes animal models provide a crucial framework for comprehending the biochemical, cellular, and molecular processes that underlie the disease‘s progression and assessing possible treatment approaches. It is well known that diabetic rat models induced by STZ can replicate human diabetic pathophysiology, especially in terms of organ damage caused by oxidative stress [10–11]. Following pharmacological intervention, these models provide controlled assessment of liver function, structural changes, and therapeutic response. Examining DHDA in these models could help close important knowledge gaps about its molecular function and therapeutic potential.

Many diabetics still lack the best defence against end-organ damage, especially hepatic impairment, despite significant advancements in pharmacotherapy, such as insulin analogues, oral hypoglycaemic medications, and incretin-based treatments. Safety issues, side effects, or inadequate effectiveness in chronic metabolic disorders frequently limit the use of currently available hepatoprotective medications [12,13]. This emphasizes the necessity of investigating new compounds that might concurrently protect hepatic architecture, enhance metabolic balance, and control oxidative stress.

Given the growing prevalence of diabetes-related liver illness worldwide and the growing understanding that the liver is a crucial target organ in chronic metabolic dysregulation, this research is pertinent [14,15].

In conclusion, oxidative and inflammatory mechanisms caused by diabetes mellitus significantly stress the liver, resulting in increasing tissue damage and malfunction [16]. Alternative substances with protective potential must be investigated because current therapeutic approaches do not provide adequate organ protection. Due to its distinct phenolic structure and documented biological action, DHDA is an intriguing chemical that merits further research. With the overarching goal of advancing the field of hepatoprotective therapies in metabolic illnesses [17], this study aimed to evaluate the protective effects of DHDA against diabetes induced hepatic damage using a STZ-NA focusing on glycaemic control, oxidative stress modulation, liver function and, histopathological changes.

MATERIALS AND METHODS

CHEMICAL AND REAGENT

STZ 98% and NA 99.5% were procured from Sisco Research Laboratories Pvt.Ltd (SRL)-Mumbai-400 099, Maharastra , India.

DHDA were procured from Avra Laboratories Pvt.Ltd-Hyderabad-500076-Telangana -India.

EXPERIMENTAL ANIMAL

Vyas Labs, Hyderabad, Telangana-501 301, provided Swiss Albino Mice (8 weeks aged, 20–25 g) and Wistar Rats (8 weeks, 180–190 g). They were kept in the Animal Home of Pharmacology Division of College of Pharmaceutical Sciences under the optimal conditions of temperature (22°C ± 1°C) and 12/12 h light/dark cycle and they were fed with the standard food had free access of water. The ethical approval was acquired from Institutional Animal Ethics Committee (IAEC) (Ref : IAEC-15/AU-Pharm/2025-26) through the Committe for the Purpose of Control and Supervision of Experiment on Animal (CPCSEA) guideline.

STUDY OF ACUTE TOXICITY

Acute toxicity study was performed according to the OECD guidelines 423 (Acute Toxic Class Method) 4 Groups (20000, 300,50,5 mg/kg) of 3 mices were used and the starting dose level of DHDA was 2000 mg/kg. The mortality and morbidity was observed after 24 hours if any. Hence,1/10 and 1/5 of the safety dose were selected for further study [18].

INDUCTION OF DIABETES AND EXPERIMENTAL DESIGN

Diabetes was induced by STZ (pH 4,5 ; 01M in Citrate buffer) in a single intraperitoneal (ip) injection (40mg/kg), 15 min after ip administration of NA

(100 mg/kg) dissolved in normal saline. On 7th Day, The FBG were measured and for those who is equal or more than 250mg/dL were kept for further study.

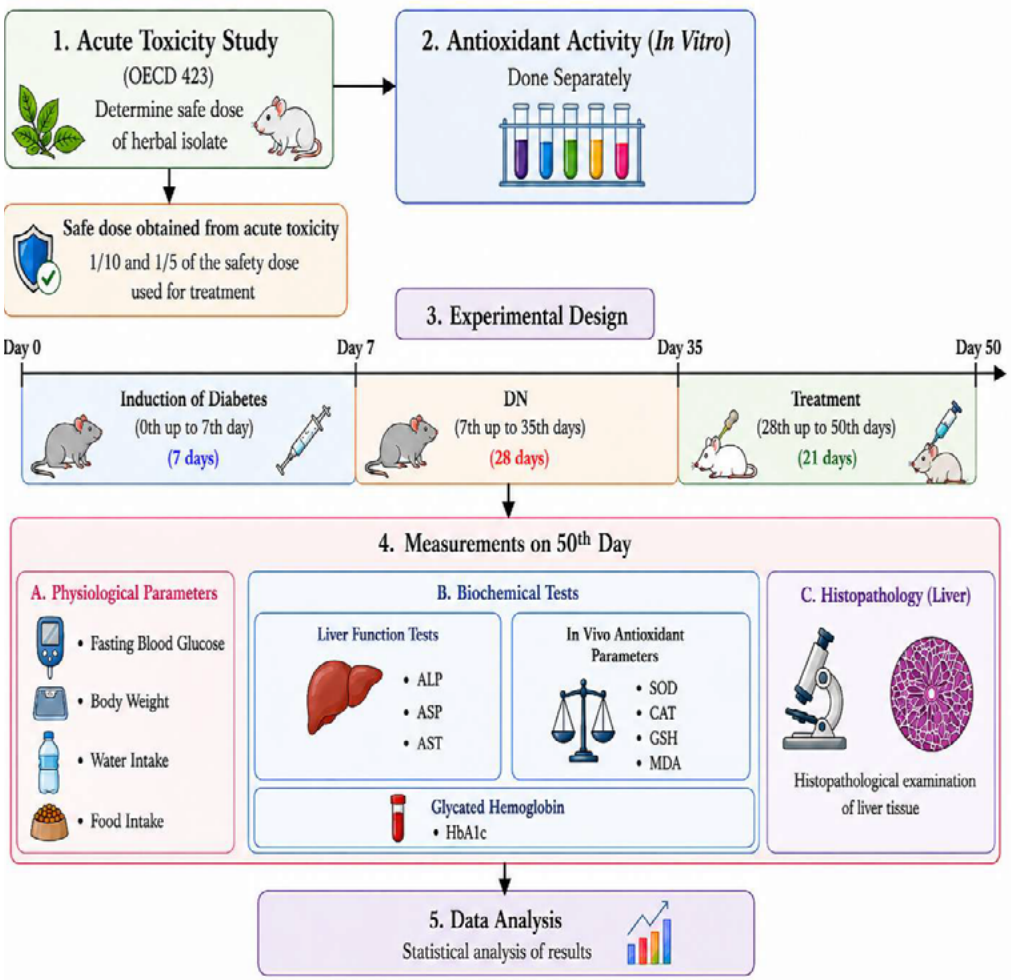
DN was developed for 28 days [19].

The animals were divided by 4 groups of 6 Rats:

Group I	Normal control which received an oral dose of carboxymethyl cellulose (CMC) (1%) solution
Group II	Disease control which received oral dose of CMC (1%) solution
Group III	Treatment with a low dose of DHDA (30 mg/kg, *po*) dissolved in CMC 1%
Group IV	Treatment with a high dose of DHDA (60 mg/kg, *po*) dissolved in CMC 1%

The treatment was started on 28th days for 3 weeks. The FBG, food intake, water intake and the body weight were monitored throughout the study .Final measurements obtained on Day 50 were used for statistical analysis and are presented in the results section. The FBG was recorded by using glucometer. At the end of the treatment (50 th day), the blood sample were collected from jugular vein under anesthesia for biochemical parameter,then, the animals were euthanized by cervical dislocation and the Liver were excised for oxidative stress markers and histopathology [19].

Figure 1: Experimental design of STZ-NA induced liver injury model



ASSESSMENT OF BIOCHEMICAL PARAMETER AT THE END OF TREATMENT (50TH DAY)

SERUM BIOCHEMICAL PARAMETER

After blood collection, the serum was separated and analyzed for estimation of HbA1C, the liver function such as Alkaline phosphate (ALP), Alanine transaminase (ALT) as well as Aspartate transaminase (AST) were also measured.

ESTIMATION OF CATALASE (CAT) ACTIVITY

CAT activity was manually recorded in Liver tissue according to cohen et al [20].

ESTIMATION OF SUPEROXIDE DISUMUTASE (SOD) ACTIVITY.

The recommended procedure for evaluating superoxide dismuase (SOD) activity is performed according to Xican Li [21].

ESTIMATION OF REDUCED GLUTATHIONE (GSH) LEVELS

GSH levels were assessed according to the Ellman's method, where GSH reacts with 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) to generate a yellow-colored product measurable at 412 nm [22].

ESTIMATION OF LIPID PEROXIDATION (LPO) LEVELS

Lipid peroxidation, serving as a marker of Liver damage caused by reactive species; was evaluated using the thiobarbituric acid reactive substances (TBARS) method [23].

IN VITRO ANTIOXIDANT ASSAYS

SUPEROXIDE RADICAL SCAVENGING ACTIVITY

The superoxide anion radical scavenging activity of extract was evaluated by using the riboflavin -light NBT.[24].

HYDROGEN PEROXIDE SCAVENGING ACTIVITY

Th Hydrogen peroxide activity was determined by method of Ruch and colleagues [25].

HISTOPATHOLOGY EXAMINATION

After removing the Liver tissue, it was washed with normal saline and fixed in 10% of Buffered formalin. They were embedded in paraffin, stained in **haematoxylin** and eosin (H and E) and observed under light microscope for histopathological change (26].Histopathological evaluation was performed by a single observer who was blinded to the treatment allocation of the experimental groups.

The scores for histopathological parameters for liver can be explained as follows [27]:

Score 0 = no visible cell damage; Score 1 =focal cells damage on < 30% of the tissue; Score 2 = focal cells damage on 30-50% of the tissue; Score 3 = extensive, but focalized cell lesions, more than 50%; and Score 4 =global cellular necrosis.

STATISTICAL ANALYSIS:

The data were analyzed using Graph Pad Prism 9 software.

The results were expressed as mean ± SEM (n = 6). Kruskall-Wallis test and One-way analysis of variance (ANOVA) followed by Tukey's test and Dunn's test as a post hoc analysis. For comparisons to the normal group, the symbols indicate: $P<0.05^{a}$. For comparisons to the disease control group, it indicate : $P<0.05^{b}$, $P>0.05^{ns}$ (non significant).

RESULTS

ACUTE TOXICITY STUDY

In this research work, n**o signs of toxicity or mortality were observed** at a the dose of 300mg/kg. The acute oral toxicity in mice showed that at a dose of 300mg/kg, the phenolic compound DHDA is safe. Hence, 1/5 th (60mg/kg) and 1/10th (30mg/kg) of this dose were selected for further study.

EFFECT OF DHDA ON BODY WEIGHT, FOOD INTAKE AND WATER INTAKE ON 50TH DAY

At the end of the treatment, the disease control group was significantly decreased ($P<0.05^{a}$) compared to the normal control group in the body weight. However, DHDA treatment at a dose of 30 mg/kg and 60 mg/kg did not show any significant improvement ($P>0.05^{ns}$) compared to the disease control group (Table 1) .

Similarly, the food intake was markedly reduced in the diabetic control group ($P<0.05^{a}$) compared to the normal control group. However, DHDA treatment at a dose of 30 mg/kg did not show a statistically significant ($P>0.05^{ns}$) but significant at dose of 60mg/kg ($P<0,05^{b}$) compared to the disease control group (Table 1).

The water intake was elevated in the disease control group ($P<0.05^{a}$) compared to the normal control group. However, DHDA treatment at a dose of 30mg/kg and 60mg/kg showed a significant amelioration ($P<0.05^{b}$) compared to the disease control group (Table 1).

Table 1. Effect of DHDA on body weight, water intake and food intake on 50th day.

Groups	Body weight (g)	Water intake (mL)	Food Intake (g)
Normal Control	299.8±10.99	563.7±16.34	228± 3.27
Disease Control	187±6.84^{a}	905.5±8.728^{a}	185.5± 12.91^{a}
DHDA (30mg/kg)	224±16.19^{ns}	712±2.978^{b}	209.8± 2.10^{ns}
DHDA (60mg/kg)	210.3±5.84^{ns}	702.2±3.701^{b}	221.3± 1.85^{b}

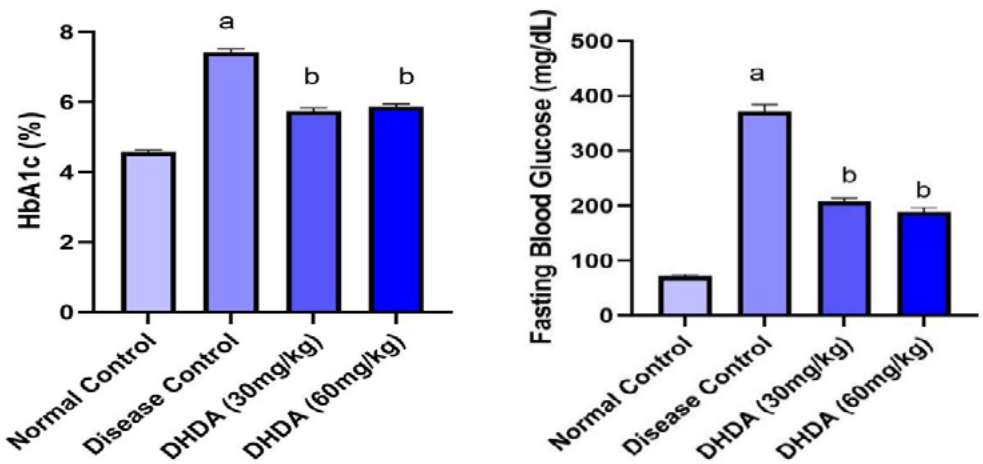
Values are presented as means ± SD (n=6). One way ANOVA was used followed by Tukey's multiple test as post hoc. Test compared to Disease control Group : $P<0.05^{b}$; compared to Normal Control Group: $P<0.05^{a}$. $P>0.05^{ns}$.(non significant)

EFFECT OF DHDA ON FBG AND HBA1C ON 50TH DAY

The FBG was significantly increased in the disease control group ($P<0.05^{a}$) compared to the normal control group. Nevertheless, the DHDA treatment at a dose of 30mg/kg and 60mg/kg showed a significant decrease ($P<0.05^{b}$) compared to the disease control group (Figure 2).

The HbA1c was elevated in the disease control group ($P<0.05^{a}$) compared to the normal control group. The treatment DHDA with both doses resulted a significant amelioration ($P<0.05^{b}$) compared to the disease control group (Figure 2).

Figure 2: Effect of DHDA on FBG and HbA1c on 50th day.



Values are presented as means ± SD (n=6). One way ANOVA was used followed by Tukey's multiple test as post hoc. Test compared to Disease control Group : $P<0.05^{b}$; compared to Normal Control Group: $P<0.05^{a}$. $P>0.05^{ns}$ (non significant).

EFFECT OF DHDA IN BIOCHEMICAL PARAMETER ON 50TH DAY

EFFECT OF DHDA ON LIVER FUNCTION ON 50TH DAY

The levels of ALP, AST showed a significant increase in the disease control group ($P<0.05^{a}$), while the ALT levels was unchanged compared to the normal control group ($P>0.05^{ns}$). The DHDA treatment at both doses resulted a significant decrease in ALP and AST levels ($P<0.05^{b}$) compared to the disease control group. However, the levels of ALT were non-significantly changed ($P>0.05^{ns}$) (Table 2).

Table 2: Effect of DHDA in ALP, ALT and AST on 50th day

Animals	ALP(IU/L)	ALT (IU/L)	AST (IU/L)
Normal Control	197.7±3,83	88.17±4.66	183.8±4,27
Disease Control	584.5±36,29^{a}	102.2±2.84^{ns}	220.2±4,58^{a}
DHDA (30mg/kg)	234±11.93^{b}	95.5±3.70^{ns}	195.5±3,25^{b}
DHDA (60mg/kg)	231.8±15.31^{b}	90.67±3.38^{ns}	189±3.27^{b}

Values are presented as means ± SD (n=6). One way ANOVA was used followed by Tukey's multiple test as post hoc. Test compared to Disease control Group : $P<0.05^{b}$; compared to Normal Control Group: $P<0.05^{a}$. $P>0.05^{ns}$ (non significant)

EFFECT OF DHDA ON OXIDATIVE STRESS IN LIVER ON 50TH DAY

MDA concentration in liver increased significantly ($P<0.05^{a}$) in the disease control group; while, the activity of CAT and SOD as well as the GSH levels were significantly ($P<0.05^{a}$) decreased compared to the normal control group. DHDA treatment at a dose of 60 mg/kg show a significant ($P<0.05^{b}$) amelioration with decrease of MDA levels and rise in CAT, GSH and SOD level compared to the disease control group. Otherwise, DHDA treatment at 30 mg/kg did not show any amelioration ($P>0.05^{ns}$) in the activity of CAT and SOD as well as the GSH levels while; MDA concentration was significantly ($P<0.05^{b}$) ameliorated compared to the disease control group (Table 3).

Table 3: Effect of DHDA on oxidative stress on 50th day

Groups	CAT (Unit/mL)	SOD (Unit/mL)	GSH (umol/g of tissue)	MDA (umol/g of tissue)
Normal Control	14.62±1,38	5.58±0,52	2.148±0,05	0.48±0.02
Disease Control	8.65±0,82^{a}	2.81±0,42^{a}	1.47±0,05^{a}	0.96±0.03^{a}
DHDA (30mg/kg)	11.74±0,85^{ns}	4.45±0,43^{ns}	1.57±0.05^{ns}	0.79±0.02^{b}
DHDA (60mg/kg)	13.87±0,62^{b}	5.16±0,44^{b}	1.61±0.02^{b}	0.79±0.03^{b}

Values are presented as means ± SD (n=6). One way ANOVA was used followed by Tukey's multiple test as post hoc. Test compared to Disease control Group : $P<0.05^{b}$; compared to Normal Control Group: $P<0.05^{a}$. $P>0.05^{ns}$ (non significant)

IN VITRO ANTIOXIDANT STUDIES

EFFECT OF DHDA ON SUPEROXIDE RADICAL

The superoxide radical scavenging activity of DHDA increased with increasing the concentration. At 500 µg/mL, the percentage of inhibition of DHDA (77.75±0.38) was slightly lower than the Ascorbic acid (AA) (85.72±0.95). The IC50 value of DHDA as 41.49 µg/mL while 41.81 for the AA (Table 4). The regression coefficient (r^2= 0.98) of DHDA confirmed an excellent linear relationship between concentration and percentage inhibition, indicating dose-dependent antioxidant efficiency (Table 4).

Table 4: Effect of DHDA on superoxide radical

Concentration (µ/mL)	DHDA	AA
100	61.46±0.74	65.58±1.19
200	65.8±10.68	67.97±0.31
300	71±1.32	74.28±1.48
400	74.9±1.32	80.65±0.87
500	77.75±0.38	85.72±0.95
r^2	0.98	0.99
IC50	41.49	41.81

Each value represents mean ± SEM (n=3)

EFFECT OF DHDA ON HYDROGEN PEROXIDE SCAVENGING ACTIVITY

DHDA showed a concentration-dependent scavenging effect of hydrogen peroxide. At 10 µg/ml, the percentage of inhibition of DHDA (58.92) was lower than the AA (89.52). The IC50 value of DHDA and AA were 8.527 and 2.75 (table 5). The regression coefficient (R2=0.98) confirms the strong link between concentration and percentage inhibition demonstrating a powerful hydrogen peroxide scavenger (table 5).

Table 5: Effect of DHDA on hydrogen peroxide scavenging activity

Concentration (µ/mL)	DHDA	AA
1	12.81±0,24	24.6±0.42
2	15.67±0.44	38.1±2.34
4	25.41±3.10	58.1±0.27
6	33.7±0.23	72.38±0.27
8	49.61±0.44	82.86±0.27
10	58.92±3.04	89.52±0.27
r^2	0.98	0.95
IC50	8.527	2,75

Each value represents mean ± SEM (n=3)

HISTOLOGICAL CHANGES IN LIVER

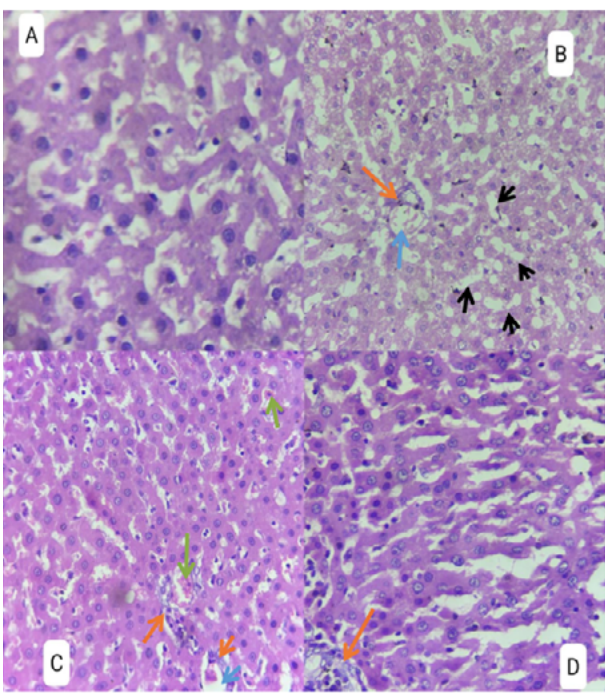
The disease control group exhibited a marked elevation ($P<0.05^{a}$) in the histopathological alteration, including necrosis, fatty degeneration, sinusoidale dilatation and hepatocyte degeneration when compared to the normal control group. Administration, of DHDA treatment at a dose of 60mg/kg produced a significant improvement ($P<0.05^{b}$) in these liver injury compared to the disease control group. In contrast, DHDA treatment at a dose of 30 mg/kg produced only mild change, which not reach statistical significance ($P>0.05^{ns}$) (table 6) (figure 3).

Table 6: Histological injury score in liver

Injury total score				
Groups	Necrosis	Fatty degeneration	Sinusoidal dilatation	Degenerated Hepatocyte
Normal control	0.4±0.24	0.4±0.24	0.2±0.2	0±0
Disease control	2.2 ± 0.2^{a}	2.6 ± 0.24^{a}	2.8 ± 0.2^{a}	3 ± 0^{a}
DHDA (30mg/kg)	1.8 ± 0.2^{ns}	1.8 ± 0.37^{ns}	1.6 ± 0.24^{ns}	1.4 ± 0.24^{ns}
DHDA (60mg/kg)	1 ± 0^{b}	1 ± 0^{b}	0.8 ± 0.2^{b}	0.8 ± 0.2^{b}

Values are presented as means ± SD (n=6).Kruskal-Wallis test was used followed by Dunn's test as post hoc. Test compared to disease control Group : $P<0.05^{b}$; compared to normal Control Group: $P<0.05^{a}$. $P>0.05^{ns.}$(non significant)

Figure 3: Histological change in liver (x10) : (A) Normal control; (B) Disease control; (C) DHDA treatment at dose 30mg/ kg; (D) DHDA treatment at dose 60 mg /kg



fatty degeneration
necrosis
Degenerated hepatocyte
sinusoidale dilatation

DISCUSSION

Diabetes mellitus is a chronic metabolic disorder with a rapidly increasing global burden, and persistent hyperglycaemia is now well recognized as a driver of oxidative stress, inflammation and progressive organ damage, particularly in the liver [28]. In the present study, diabetes was induced using the STZ–NA model, which is widely accepted as an experimental analogue of type 2 diabetes. NA partially protects pancreatic β-cells from complete destruction by STZ, resulting in moderate insulin deficiency and chronic hyperglycaemia rather than absolute insulin lack, as described by Szkudelski and Masiello [29,30].

In line with **the findings** by Etuk and by Masiello et al, the diabetic control animals in our study showed marked elevations in FBG and HbA1c, together with classical clinical features

such as anorexia, polydipsia and altered body weight [31,32]. Treatment with DHDA produced a clear antihyperglycaemic effect. Both 30 mg/kg and 60 mg/kg doses significantly lowered FBG and HbA1c compared with untreated diabetic rats, suggesting improvement in long-term glycaemic regulation rather than a transient fall in blood sugar. This pattern is comparable to the glucose-lowering actions of caffeic acid, cinnamon extract and quercetin reported by Subash Babu et al. and Vessal et al., respectively, in STZ-induced diabetic rats [33,34,35]. In those studies, the phenolic compounds not only reduced blood glucose but also attenuated oxidative stress and improved liver biochemistry. Similarly, Singh et al. showed that chlorogenic acid improved glycaemic status and hepatic markers in diabetic animals [36]. Taken together, these data position DHDA within the broader class of phenolic agents with dual antidiabetic and organ-protective properties. Despite this significant correction in glycaemic indices, DHDA did not completely normalize body weight, which remained lower than in non-diabetic controls. A similar dissociation between metabolic parameters and body weight has been reported by Kim et al study which observed improvement in glucose control and oxidative stress with polyphenol treatment in diabetic rats without full restoration of body weight over the treatment period [37]. In our study, the reduction in excessive water intake and better regulation of food intake particularly at 60 mg/kg indicate a partial correction of catabolic and osmotic symptoms, even in the absence of full weight recovery. This suggests that a longer treatment duration or combination therapy may be required to reverse established weight loss.

Biochemical evidence of hepatic involvement in the diabetic state was demonstrated by elevated AST and ALP in the disease control group. These enzyme changes reflect hepatocellular injury and disturbed bile canalicular function, which are well documented in diabetic hepatopathy [37]. DHDA significantly reduced AST and ALP, especially at the higher dose, indicating hepatoprotection. Comparable findings have been reported with other antioxidants. Al-Enazi study demonstrated that polyphenols reduced liver enzymes and improved histology in diabetic rats, while Singh et al. found that chlorogenic acid protected hepatic tissue from STZ-induced damage [36]. Aldahmash et al study showed that biotin supplementation in STZ-induced diabetic mice lowered ALT and AST and improved histopathological scoring, supporting the concept that antioxidant vitamins and phenolic molecules can attenuate diabetes-related hepatotoxicity [38]. In our study, ALT remained statistically unchanged, which may indicate that the primary lesion was oxidative and microvascular rather than extensive confluent necrosis, a pattern also observed by Hamadi et al. in STZ-induced liver injury [39].Moreover, AST is not exclusively liver-specific and may also comes from extrahepatic tissues, whereas elevated ALP may reflect alterations in biliary canalicular function or cholestatic changes associated with diabetes [40]. Therefore, the concurrent elevation of AST and ALP, together without significant ALT changes, suggests that the hepatic injury in our model may involve mild hepatocellular stress accompanied by biliary dysfunction rather than severe hepatocyte membrane disruption [41]. The reduction of AST and ALP following DHDA treatment nevertheless supports an improvement in diabetes-associated hepatic and hepatobiliary alterations.

Oxidative stress is a central mechanism linking hyperglycaemia to tissue injury. Valko et al. emphasized that prolonged ROS generation and impaired antioxidant defence contribute directly to cellular damage in chronic diseases including diabetes [42]. In our study, diabetic animals exhibited significant depletion of SOD, CAT and GSH, accompanied by increased MDA levels, confirming increased lipid peroxidation and impaired redox balance.The decrease in GSH levels represents increased utilization due to oxidative stress while The decline in CAT and SOD activities observed in diabetic animals may be attributed to increased oxidative stress caused by the overproduction of H_2O_2 and superoxide radicals through glucose autoxidation and protein glycation reactions [43]which leads to the lipid peroxidation by taking an electron

from polyunsaturated fatty acid and proceed to the formation of MDA [44,45]. DHDA, especially at a dose of 60 mg/kg, restored antioxidant enzyme activities and markedly reduced MDA, may be due to the free radical scavengng activity of DHDA. Also, this restoration indicates potent antioxidant action in vivo . This is consistent with the study by Bahadoran et al., which showed that dietary polyphenols improve antioxidant status and insulin sensitivity in diabetic subjects and models [46], and with the mechanistic insights of Dai & Mumper study which described how phenolic compounds upregulate endogenous antioxidant defences and scavenge free radicals [47].

Our *in vitro* experiments further support the antioxidant capacity of DHDA. The compound showed a strong superoxide scavenging activity with an IC50 comparable to ascorbic acid, along with dose-dependent hydrogen peroxide scavenging. Rezk et al. demonstrated that 2',6'-dihydroxyacetophenone is a key antioxidant pharmacophore within certain dihydrochalcones, providing efficient radical stabilisation and explaining their strong antiperoxidative effects [48]. The close agreement between our *in vitro* and *in vivo* findings suggests that DHDA's direct radical-neutralizing ability contributes substantially to its hepatoprotective and antihyperglycaemic effects.

Histopathological observations in the present study are also in accordance with previous reports. In our study, diabetic control livers showed hepatocyte degeneration, fatty change, sinusoidal dilatation and necrotic areas, similar to the severe lesions described by Aldahmash et al. in STZ-induced diabetic mice, where ballooning degeneration, steatosis and inflammatory infiltrates were prominent [38]. Treatment with DHDA, particularly at a dose of 60 mg/kg, markedly improved hepatic architecture and reduced injury scores. This tissue-level recovery resembles the hepatoprotective effects of naringenin reported by Kapoor and Kakkar study, which demonstrated that this flavanone reduced oxidative stress, normalised mitochondrial function and attenuated apoptotic signalling in the livers of STZ-induced diabetic rats. Aboonabi and co-workers likewise showed that pomegranate seed juice improved antioxidant status and ameliorated hepatic histological changes in STZ NA diabetic rats, reinforcing the concept that phenolic-rich interventions can restore both biochemical and structural integrity in diabetic liver injury [49,50].

Overall, when our findings are viewed alongside the published work on caffeic acid, quercetin, naringenin, chlorogenic acid, biotin and pomegranate polyphenols. DHDA demonstrates a comparable profile: significant antihyperglycaemic action, strong antioxidant effects, improvement in liver enzymes and histology, and partial renal protection. The pronounced effects at a dose of 60 mg/kg, in particular, suggest a dose–response relationship. Future studies should focus on defining the molecular targets of DHDA (for example, its influence on NF-κB, mitochondrial pathways and insulin signalling), exploring chronic administration, and evaluating combination strategies with standard antidiabetic drugs. Such work will be crucial to determine whether DHDA can progress from an experimental phenolic compound to a viable adjunct in the management of diabetes-associated hepatic and metabolic complications.

CONCLUSION

The study reveals that DHDA exerts considerable hepatoprotective and antioxidant effects in STZ-NA induced diabetic rats. Improvements in biochemical indicators, oxidative stress markers, glycemic control, and histological findings indicate that DHDA may serve as a promising treatment candidate for controlling diabetes-associated liver injury, with 60 mg/kg displaying the most prominent protective impact.

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